First report of grapevine Rupestris stem pitting-associated virus in wild grapevines (*Vitis vinifera* spp. *sylvestris*) in Tunisia

I. Selmi, Laboratoire de Protection des Végétaux, Institut National de la Recherche Agronomique de Tunisie, Rue Hedi Karray, 1004 ElMenzah, Tunis, Tunisie ; **D. Pacifico,** Istituto di Bioscienze e BioRisorse (IBBR), Consiglio Nazionale delle Ricerche- CNR Corso Calatafimi 414, I-90129 Palermo – Italia ; **M. Harbi Ben Slimane,** Laboratoire d'Horticulture, Institut National de la Recherche Agronomique de Tunisie, Rue Hedi Karray, 1004 ElMenzah, Tunis, Tunisie ; **G. Garfi** and **F. Carimi,** Istituto di Bioscienze e BioRisorse (IBBR), Consiglio Nazionale delle Ricerche- CNR Corso Calatafimi 414, I-90129 Palermo – Italia ; and **N. Mahfoudhi,** Laboratoire de Protection des Végétaux, Institut National de la Recherche Agronomique de Tunisie, Rue Hedi Karray, 1004 ElMenzah, Tunis, Tunisie.

Wild grapevines (*Vitis vinifera* spp. *sylvestris*) grow in the northern part of Tunisia, and can potentially be natural reservoirs of pathogens including viruses. Grapevine Rupestris stem pitting-associated virus (GRSPaV), a member of the genus *Foveavirus* in the family of *Betaflexiviridae*. It is present in grapevines worldwide and is associated with rupestris stem pitting (RSP) and grapevine vein necrosis (Meng et al. 2013). The virus has been detected in the pollen of infected grapevines (Rowhani et al. 2000), but its spread through pollen is not confirmed, although it is transmitted by seed from infected mother plants to their progeny (Lima et al. 2006b). In Tunisia, GRSPaV is very common in table grape cultivars (Soltani et al. 2013) but no data are currently available on the presence of viruses in Tunisian wild grapevines, which can play a role in the dissemination of viruses to the cultivated grapevines. To address this knowledge gap, a survey was carried out in the mountain forests of northern Tunisia. Samples of wild grapevines were labeled during the vegetative season and dormant canes from 84 accessions (male and female plants) were collected during winter. All samples were tested by RT-PCR for the presence of GRSPaV using primers RSP-48 (5'-AGCTGGGATTATAAGGGAGGT-3') and RSP-49 (5'- CCAGCCGTTCCACCACTAAT-3') (Lima et al. 2006a) for the amplification of a 331 bp fragment of the coat protein (CP) gene. Results showed that 51% (43/84) of the samples were infected by GRSPaV. In order to confirm the presence of this virus in wild grapevines, two positive samples (VS56 and VS70) were tested by RT-PCR using primers RSP-52 (5'-TGAAGGCTTTAGGGGTTAG-3') and RSP-53 (5'-CTTAACCCAGCCTTGAAAT-3') (Rowhani et al. 2000) to amplify the complete CP. Isolate VS56 was from a male plant in northern Tunisia and isolate VS70 was from a female plant in the northeast of the country. PCR products of these two isolates were

cloned and sequenced in both directions. The Tunisian GRSPaV isolates VS56 (LT855232) and VS70 (LT855235) shared 84% nucleotide sequence identity. Isolate VS56 had 85-86% identity with all GRSPaV sequences available in GenBank, whereas VS70 showed 93-99% identities with isolates SK704-A (KX274274) and ORPN12 (FJ943318). To further confirm the presence of GRSPaV in wild grapevines, the same two samples were tested by RT-PCR using primers McK1U (AGGGATTGGCTGTTAGATGTT) and McK1D (CTTCAGGCAACCCCAAAAA) (Nolasco et al. 2000) to amplify a 355 bp fragment of the RNA-dependent RNA polymerase domain. Isolates VS56 (LT906626) and VS70 (LT906636) shared 89% nucleotide sequence identity. Isolate VS56 had 89-94% identity with isolates SK30 (KX274277) and GRSPaV-MG (FR691076) while VS70 showed 94-95% identity with isolates Tannat-Rspav1 (KR528585) and GRSPaV-GG (JQ922417). To our knowledge, this is the first report of GRSPaV in wild grapevines in Tunisia.

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